Molecular Biology

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Title:	**G Protein Suppressor Screens and Analysis of Gene Expression in *Neurospora crassa***

Heterotrimeric G proteins are essential for the response to hundreds of environmental signals in eukaryotic cells. The three central components of G proteins are α, β, and γ subunits. Dr. Katherine Borkovich's lab has previously identified two Gα subunit genes, *gna-1* and *gna-2*, from the filamentous fungus *Neurospora crassa*. Deletion of *gna-1* leads to defects in differentiation of asexual spores, apical extension, sensitivity to hyperosmotic media, and female fertility. Deletion of *gna-2* in a Δ*gna-1* background leads intensification of phenotypes. Furthermore, Δ*gna-1* strains are more resistant to heat while strains with the activating mutation *gna-1*^{Q204L} are more sensitive to heat.

Hyperosmotic sensitive strains Δ*gna-1* Δ*gna-2* and heat sensitive *gna-1*^{Q204L} strains were used to screen for suppressor mutations. In hyperosmotic sensitivity screens, the growth rate of the Δ*gna-1* Δ*gna-2* strains was observed to be identical on sorbose medium while it was selective on minimum medium. In the heat sensitivity screens, the *gna-1*^{Q204L} strains were observed to have actually the same survival rate as the wild-type strain on medium with sorbitol. The proposed conclusion of the suppressor screens was that hyperosmotic medium increases thermotolerance of the heat-shocked *gna-1*^{Q204L}, while suppressing the survival rate of wile type.

In addition to suppressor screens, Northern Blot analysis was used to determine the expression pattern of certain genes in various G protein mutant strains. The candidate genes probed included those for heat shock proteins (hsp), catalase, mating type, GAPDA, and a MAP kinase. The hsp83 had various expression levels in all the mutant strains and an especially high expression level in *cr-1* and *gna-3* strains. All the mutant stains also showed some expression level for the gpd-1 gene.